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Composition of Alginates from Brown Seaweeds, *Sargassum* and *Padina* spp.

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ABSTRAK

Peratus kandungan alginat dalam rumpair coklat, Sargassum spp. *dan* Padina spp., *dari Sabah, serta kelikatan intrinsik sampel-sampel alginat berkenaan telah ditentukan.* Sargassum *yang muda dan yang matang masing-masing memberi peratus hasil sebanyak 35% dan 32%, sementara* Padina *hanya memberi 18.5% hasil alginat. Kelikatan intrinsik, [η], yang didapati bagi alginat daripada* Sargassum *matang, dan* Padina *masing-masing ialah 412 mL/g dan 312 mL/g. Kajian menunjukkan bahawa kelikatan intrinsik larutan alginat dipengaruhi oleh sumber dan spesies rumpair, kepekatan garam pelarut, serta kaedah pengekstrakannya. Alginat yang telah dirawat dengan larutan NaOCl mempunyai kelikatan intrinsik yang rendah, iaitu [η] = 210 mL/g. Sementara alginat yang mempunyai kelikatan intrinsik lebih tinggi, [η] = 650 mL/g, telah dihasilkan oleh sampel-sampel* Sargassum *yang telah direndam dalam larutan formaldehid cair. Hasil daripada penentuan kelikatan intrinsik juga digunakan bagi penentuan berat molekul kelikatan* M_v, *alginat menggunakan persamaan Mark-Houwink. Komposisi blok dalam sampel alginat telah ditentukan dengan menggunakan kaedah hidrolisis heterogen separa. Hasil menunjukkan bahawa* Sargassum *muda mempunyai alginat dengan kandungan poli (β-D-mannuronat) yang lebih tinggi, tetapi* Sargassum *matang mempunyai peratus kandungan poli (α-L-guluronat) yang lebih tinggi. Nisbah M/G yang telah ditentukan bagi sampel-sampel alginat tersebut ialah 1.27, 0.64 dan 0.85, bagi* Sargassum *muda,* Sargassum *matang dan* Padina. *Ini menunjukkan bahawa* Sargassum *matang dapat menghasilkan alginat yang mempunyai nisbah M/G terendah.*

ABSTRACT

The percentage yield of alginate in brown seaweeds, Sargassum *and* Padina *spp., from Sabah, and the intrinsic viscosities of these alginates were determined. Young and mature* Sargassum, *respectively, gave 35% and 32%, while* Padina *only yielded 18.5% of alginate. The intrinsic viscosities [η], obtained were 412 mL/g and 312 mL/g for alginates from mature* Sargassum *and* Padina, *respectively. It was found that these intrinsic viscosities depend on the source and species of seaweeds. Treating the alginate sample with bleaching agent such as NaOCl yielded samples with [η] = 210 mL/g. However, a higher intrinsic viscosity, [η] = 650 mL/g, was obtained from* Sargassum *samples which were pre-treated in dilute formaldehyde solution. Results from intrinsic viscosity determination were used to estimate the viscosity average molecular weight,* M_v, *of the phycocolloid using the Mark-Houwink equation. Block compositions of alginates were determined using the technique of partial heterogenous hydrolysis. Results showed that young* Sargassum *was richer in the poly (β-D-mannuronate) block, while matured* Sargassum *was richer in the poly (α-L-guluronate) block. From these compositions, M/G ratios were estimated as*

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1.27, 0.64 and 0.85 for alginates from young Sargassum, *mature* Sargassum *and* Padina, *respectively. This shows that mature* Sargassum *yields alginates with the lowest M/G ratio.*

INTRODUCTION

Alginate is the major structural polysaccharide found in brown seaweeds (Phaeophyceae), which represent the commercial source from which alginates are extracted for use as gelling agents and stabilizers in food and other industries (Overeem, 1979; Whistler, 1973). However, certain species of bacteria, such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa,* were also reported to produce alginates (Larsen and Haug, 1971).

Chemically, alginate is a (1→4) linked, linear copolymer of α-L-guluronate and β-D-mannuronate arranged in block structures (Haug and Larsen, 1962; Penman and Sanderson, 1972). The structures consist of homopolymeric buckled ribbons of poly (α-L-guluronate) which play an important role in the calcium ion mediated gelation mechanism (Morris *et al.,* 1978), and poly (β-D-mannuronate), together with sequence that approximate to alternating guluronate-mannuronate repeating units (Haug *et al.,* 1967; Boyd and Turvey, 1978), which are not involved in gelation.

The relative portions of these residues and their sequences within the primary structure vary from species to species (Haug *et al.,* 1974; Strockton *et al.,* 1980). Gel properties depend on these block structures and the poly (β-D-mannuronate)/poly (α-L-guluronate) or M/G ratio. The lower the M/G ratio, the stronger and more brittle is the gel that is formed (Penman and Sanderson, 1972). Gelation occurs through the formation of "egg-box" structures by the poly (α-L-guluronate) blocks which strongly chelate Ca^{2+} (Morris *et al.,* 1978).

In view of the fact that different species of brown seaweeds produce alginates of different compositions and structures (Haug *et al.,* 1974), it is important to characterise alginate from a new source of seaweeds, in order to evaluate its commercial potential as stabilizers and gelling agents. In this study we investigated the uronic acid compositions and viscosity of alginates isolated from *Sargassum* spp. and *Padina* spp. from Sabah, Malaysia. *Sargassum* is the major species of brown seaweeds found growing along the rocky coast of Sabah, while *Padina* appears to be the second most important brown seaweed in term of abundance.

Alginates were extracted from these seaweed samples by the modified method of Haug (1959), using 1% (w/v) Na_2CO_3 solution. In an earlier study we reported that alginate extracted from *Sargassum,* which was pre-soaked overnight in dilute HC1(0.1 M) prior to extraction in 1% Na_2CO_3, was shown to possess low intrinsic viscosity (Omar and Ghani, 1986).

In the present study, we investigated the effect of adding bleaching agent, sodium hypochlorite (NaOCl), to alginate extract and the viscosity property of alginate solution was investigated. Bleaching agents such as NaOCl and $Ca(OCl)_2$ are often employed in industrial preparation of alginates (Chapman and Chapman, 1980). Seaweed samples were also treated by soaking overnight in dilute aqueous formaldehyde solution, prior to extraction in 1% Na_2CO_3 solution, in order to remove phenolic compounds. The effect of such a treatment on alginate viscosity property was studied. The M/G ratios for alginates isolated from young and mature *Sargassum* spp. and from *Padina* spp. were compared.

MATERIALS AND METHODS

Collection of Seaweeds and Extraction of Alginates

Seaweeds samples were collected from Kuala Abai, near Kota Belud, Sabah (Malaysia), in November 1985. Samples were washed thoroughly with tap and distilled water, sun dried and ground to powder form. About 10 g of the dry sample was extracted for alginate in 250 mL. 1% (w/v) Na_2CO_3 for 24 hours at room temperature. Another 10 g of dry gound seaweed sample was similarly extracted in 3% (w/v) Na_2CO_3 solution. From each extract, a viscous brownish solution was obtained, and this was filtered on muslin cloth. Alginate was precipitated as the sodium salt by addition of approximately 3 volumes of ethanol to the aqueous solution. This was filtered by vacuum suction and washed twice with ethanol and once with diethyl ether. Sodium alginate obtained was yellowish in colour, and dried in the oven at 35–40° and its yield determined.

A 10 g of dry Sargassum sample was soaked in 30% (w/v) aqueous formaldehyde solution for 12 hours in order to remove phenolic compounds. The solvent was filtered off and the seaweed washed with distilled water, then allowed to dry in the sun. Sodium alginate was then extracted using 1% Na_2CO_3 according to the procedure described earlier.

Bleaching of Sodium Alginate Product

Seaweed extract from *Sargassum* spp. was bleached with sodium hypochlorite solution until the viscous brownish solution of sodium alginate turned to pale brown. The discoloured product was then precipitated in ethanol and treated as before.

Moisture Content

About 1 g sodium alginate prepared above was accurately weighed and dried in a vacuum oven at 70 °C for 24 hours. The sample was allowed to cool to room temperature in a vaccum dessicator and re-weighed. Moisture content of the alginate sample was determined from the weight difference and expressed as percentage of original weight.

Block Composition and M/G Ratio

Block composition of sodium alginates was determined by partial heterogenous hydrolysis (Haug *et al.*, 1974). About 1 g sample was accurately weighed and hydrolysed in 100 mL 0.3 M HCl for 2 hours at 100°C. A suspension was formed, allowed to cool to room temperature, and centrifuged at 4000 rev/min. for 2 hours. The supernatant, refered to as fraction A, was decanted off and the amount of alginate in the soluble fraction was measured by phenol-sulphuric acid reaction (Dubois *et al.*, 1956), using moisture known commercial samples as standard.

The residue from above was re-dissolved in a small amount of 0.1 M NaOH solution and then diluted to 50 mL by adding 0.1 M NaCl. The pH was adjusted to 2.85 by adding 0.0025 M HCl. A precipitate was formed and this was centrifuged down at 4000 rev./min. The supernatant was decanted and amounts of alginate in soluble fraction, referred to as fraction B, and in the final residue, referred to as fraction C, were determined by the phenol-sulphuric acid reaction as mentioned earlier, using pure mannuronic and guluronic acid as standard.

Intrinsic viscosity [η]

Intrinsic viscosities of sodium alginate solutions were determined in Cannon-Ubbelohde semi-micro dilution viscometer, size 75, at 20°C. The solvent used was 0.2 M NaCl, and alginate samples were dialysed to equilibrium against the solvent in cellulose dialysis sac (type 250-9U). Dialysate was used as diluent. The final concentration of alginate was determined using the phenol-sulphuric acid reaction.

RESULTS AND DISCUSSION

Table I show the percentage yield of alginates extracted from the seaweeds samples by the modified methods of Haug (1959) and the moisutre content. Values obtained for alginate extracted in 1% Na_2CO_3 were in good agreement with reports of earlier studies (Wedlock *et al.*, 1986a; Omar and Ghani, 1986). There was no significant difference in the yield of alginate extracted in 1% or in 3% Na_2CO_3 solution. However, the yield obtained from young *Sargassum* specimen was found to be slightly higher than from mature specimen. The extraction procedure used in this studies deviates from the standard method (Haug, 1959) by omitting the acid treatment; a previous report (Wedlock *et al.*, 1986a) has demonstrated that this is a valid procedure for the species investigated here. Studies showed that alginate of young *Sargassum* spp. had a lower moisture content.

Table 2 gives the block composition and M/G ratios of alginate samples. Results are expressed as percentage of alginate found in appropriate fractions, that is, alginate soluble and insoluble at the indicated pH. Fraction A, which refers to solubilised alginate fraction in boiling 0.3 M HCl, corresponds to the poly (β-D-mannuronate-α-L-guluronate) block which is the portion of alginate most labile to acidic hydrolysis. Fraction B corresponds to poly (β-D-mannuronate) block and was soluble at pH 2.85, while fraction C, the most stable portion and insoluble at pH 2.85, refers to the poly (α-L-guluronate) block. M/G ratio was calculated from the poly (β-D-mannuronate) and poly (α-L-guluronate) fractions, assuming that the alternating block (α-L-guluronate)frac-

TABLE 1

Percentage yield and moisture content of alginates from different sources, extracted with 1% Na_2CO_3.

Source of alginate	Yield (%)	Moisture (%)
Sargassum sp (mature)	32.0	20.0
Sargassum sp (mature)	33.0*	
Sargassum sp (young)	35.0	14.0
Padina sp	18.5	19.0
Ascophyllum nodosum	20–30+	
Laminaria hyperborea	20–30+	
Macrocystis pyrefera	13–24+	
Laminaria digitata	20–45+	

Note:

*extracted with 3% Na_2CO_3.

+commercial species, from Chapman and Chapman, 1980.

TABLE 2

Block compositions of uronates and M/G ratios in alginates from different sources

Source	GG-Block (%)	MM-Block (%)	MG-Block (%)	M/G
Sargassum sp (mature)	42	20	38	0.64
Sargassum sp (young)	24	36	40	1.27
Padina sp	36	28	36	0.85
Ascophyllum nodosum	14	15	61	1.25
Laminaria hyperborea	54	13	24	0.60

tions, which was solubilised during hydrolysis, has an M/G ratio of 1.0. From this study it was found that alginate from mature *Sargassum* spp. had the lowest M/G ratio with 0.64, while that from young *Sargassum* specimen the highest with M/G ratio of 1.27, and this is quite consistent with an earlier finding (Wedlock *et al.*, 1986). Alginate from *Padina* spp. had an M/G ratio of 0.85.

The actual M/G ratio in all cases might be slightly higher than results obtained by partial hydrolysis being reported here. This may be because the alternating poly (β-D-mannuronate-α-

L-guluronate) block normally has a M/G ratio greater than 1.0; the range being 1.2–1.4, due to the occurence of -MMG-and -MMMG- sequence (Haug *et al.*, 1967; Larsen, 1981). In addition, some of the poly (β-D-mannuronate) block might be solubilised during hydrolysis since they are more susceptible than the poly (α-L-guluronate) block (Haug *et al.*, 1967).

In this study it was found that the M/G ratio for alginate from young *Sargassum* spp. was about twice that of mature *Sargassum* spp. A higher M/G ratio alginate found in young seaweeds was expected since during the early stage of seaweeds development, mainly mannuronic acids make up alginate chains. Mannuronic residues are later epimerised to guluronic acid by enzyme C_5-epimerase at some later development stage (Larsen, 1981). As the algal tissues become older, more mannuronic acid being epimerised to guluronic acid and the M/G ratio of the alginate becomes smaller. The effect is to make the seaweed tissues stronger and to give the alginate a higher gel strength.

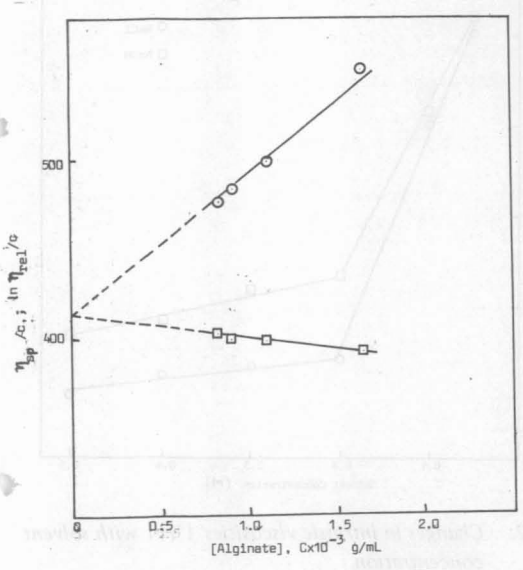

Fig. 1: Plots of η_{sp}/c (O) *and ln* η_{rel}/c (□) *versus c, where c is concentration of alginate.*

The intrinsic viscosity, $[\eta]$, of alginate was obtained by plotting values of η_{sp}/c against c, or $\ln \eta_{rel}/c$ against c, wehre η_{sp} and η_{rel} are specific and relative viscosities, respectively, and c is the alginate concentration in g/mL. These plots, on extrapolation to zero concentration, intersect at a point on ordinate to give the intrinsic viscosity, $[\eta]$. *Figure 1* shows such plots for alginate extract from mature *Sargassum* spp., where $[\eta]$ = 412 mL was obtained. Intrinsic viscosities for various alginate samples measured in 0.2 M NaCl are summarised in Table 3. It was found that alginate from *Sargassum* spp. possessed a higher intrinsic viscosity compared with *Padina* spp. Using 3% Na_2CO_3 solution as extractant resulted in a lowering of the viscosity of the alginate solution. An intrinsic viscosity of 625 mL/g was obtained for the alginate extract from *Sargassum* by the same method (Wedlock *et al.*, 1986a). However, the alginate from the same source, when extracted by precipitation with an excess $CaCl_2$ solution, was reported to possess an intrinsic viscosity of 635 mL/g (Wedlock *et al.*, 1986b). It has been suggested that the viscosity property of all alginates is affected by method of extraction (Wedlock *et al.*, 1986). Prolonging the period of extraction in higher Na_2CO_3 concentration also causes degradation of alginates.

Bleaching agents such as sodium hypochlorite and calcium hypochlorite are often used in industrial preparation of alginates, in order to improve the colour and appearance of the products. Addition of sodium hypochlorite, NaOC1, to the extract gave a clearer solution of sodium alginate, and the product obtained was pale yellow in colour. However, sodium alginate extracted using this technique was found to have a much lower intrinsic viscosity (i.e. $[\eta]$ = 210 mL/g), when compared with extraction methods which did not employ NaOC1. It has been suggested that bleaching agents such as hypochlorite, due to its ability to form free radical intermediates in solution, could enhance the degradation of alginates in solution (Fasihuddin 1987). The low $[\eta]$ could be due to such degradation process.

Our analysis of an alginate sample extract from seaweed which was pre-treated with aqueous formaldehyde, showed a much higher $[\eta]$, where a value of 650 mL/g was obtained. Phenolic compounds have been implicated with the oxidative degradation of alginates through their ability to form free radical intermediates (Fasihuddin B.A. *et al.*, 1987). Formaldehyde also kills bacteria or micro-organism in seaweeds which could contri-

TABLE 3
Intrinsic viscosity, $[\eta]$, and viscosity average molecular weights, M_v, measured in 0.2 M NaCl, for alginates from different sources and methods of extraction

Source	Extractant	mL/g	$\overline{M}_v$
Sargassum (mature)	1% Na_2CO_3 (untreated)	412	2.25×10^5
	3% Na_2CO_3 (untreated)	400	2.17×10^5
	1% Na_2CO_3 (treated with NaOCl)	210	1.05×10^5
	1% Na_2CO_3 (seaweeds pre-soaked in formaldehyde)	650	3.77×10^5
Padina	1% Na_2CO_3 (untreated)	312	1.64×10^5

bute to the degradation of alginates. The presence of transition metal ions, such as Fe^{2+} or Fe^{3+}, together with phenolic compounds would enhance free radical formation which causes depolymerisation of phycocolloids (Parson *et al.*, 1985). Therefore, removal of phenolic compounds prior to extraction in Na_2CO_3 solution reduces the degradation of alginates. Results obtained here were consistent with the higher intrinsic viscosities obtained from formaldehyde treated samples.

Table 3 also summarizes the viscosity average molecular weights, Mv, calculated using the Mark-Houwink equation $[\eta] = KM^a$ (Wedlock *et al.*, 1986) where K and a are constants with values of 8.04×10^{-3} g/mL and 0.88, respectively, for the viscosity of alginate solution measured in 0.2 M NaCl at 20°C.

Intrinsic viscosity of *Sargassum* alginate was also measured in various concentrations of NaCl and NaOH solutions, *(Figure-2)*. It was found that intrinsic viscosities increased as the solvent concentration was decreased. $[\eta]$ equals to 520 and 400 mL/g were obtained in 0.05 M NaCl and 0.5 M NaCl, respectively. A similar trend occurred when viscosities were measured in dilute NaOH, where $[\eta]$ equals to 526 and 420 mL/g were obtained in 0.05 M NaOH, and 0.50 M NaOH, respectively and this can be regarded as electroviscous effect

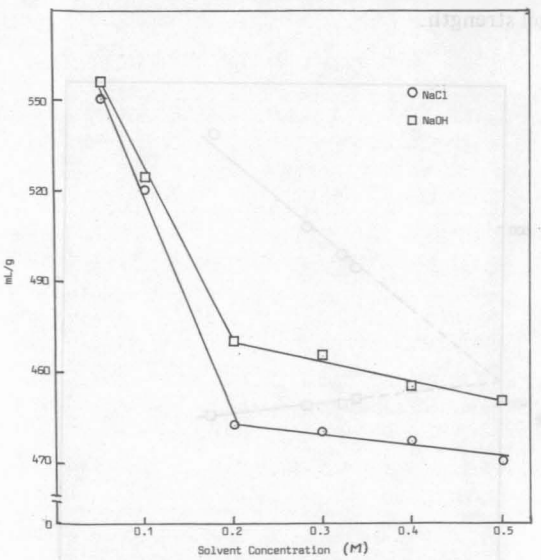

Fig 2: Changes in intrinsic viscosities $[\eta]$, with solvent concentration.

because alginates behave as a typical polyelectrolyte.

CONCLUSION

In conclusion, it has been shown that mature *Sargassum* spp. obtained locally yielded quite a high quality alginate. This was based on its low

M/G ratio, which is essential for the Ca^{2+} - mediated gel formation of strong and brittle gel. It was also shown that the industrial method which employs bleaching agents, such as sodium hypochlorite, in order to improve the appearance of the product, produced alginates of low molecular weight average, based on intrinsic viscosities meassurement. Pretreatment of seaweed samples with a dilute formaldehyde solution gave alginates of high molecular weight average.

Alginates obtained from *Padina* had a lower molecular weight average than that from *Sargassum*. These studies also showed that Sargassum spp. which are found in abundance in Sabah intertidal waters, could become a commercial source of alginate.

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